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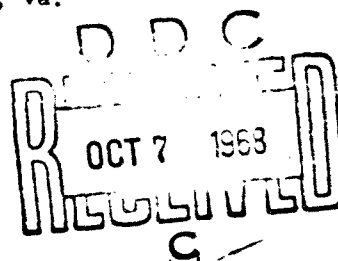
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## CONCERNING THE INTERACTION OF ANTHRAX BACILLI AND TISSUE CULTURES

[Following is the translation of an article by A.F. Safronov, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 10, 1966, page 145. It was submitted on 9 March 1966. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The attention of investigators is being drawn all the more to the use of tissue cultures as biological models for the study of various problems on the interaction of pathogenic bacteria and their toxins with the living cell. The conclusion can be drawn from recently published papers that virulent strains of pathogenic bacteria, in contrast to strains with an attenuated virulence and saprophytes, cause the death of tissue cultures. In this connection our attention was drawn to the work by Ginsburg, et al. (1963, 1964), in which they point out that virulent anthrax bacilli, in contrast to bacilli with a sharply attenuated virulence (STI-1 vaccine), exerted a cytopathic effect on human embryo cells.

In the present report data is presented which was obtained from the infection of transplantable cells of human amnion and HEp2 cells with highly virulent anthrax bacilli. For a comparison tests were set up with the vaccine strain and one of the strains of avirulent anthracoids. The tissue culture was incubated by the generally accepted method on medium No. 199 with 10% donor serum. The bacterial cultures were incubated for 24 hours on meat-peptone agar, emulsified in physiological solution, and diluted to that concentration at which following infection of the tissue cultures there would be 1 million cells in each test tube. Before the tests were set up the nutrient medium was removed, the cells washed with physiological solution and then medium No. 199 added. The infected cultures were placed in an incubator (37°). The controls were noninfected cell cultures and cells in nutrient medium into which only physiological solution was added. The cultures were studied under a microscope at a small magnification every three hours. Microscope slides were inserted into part of the test tubes. When changes were detected in the cell monolayer they were withdrawn, carefully washed with physiological solution, fixed with methyl alcohol and used in the capacity of preparations. Each test was repeated 5 times.

The investigations showed that following the infection of tissue cultures with a vaccine strain and anthracoids, the disengagement of the cells from the glass and their death set in by the end of the 2nd day of observation, probably as a result of the growth of the stated strains in the nutrient medium, its weakening, change of pH, etc. We were not

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successful in revealing intracellular arrangement of the bacilli and changes in them. A completely different effect was exerted by the anthrax bacilli: Already in 9 hours, with the absence of growth in the nutrient medium, clearly expressed changes in the cells set in - upon microscopic examination of stained preparations it was distinctly apparent that the chains of anthrax bacilli, as if intergrowing between the cells, broke down the intercellular lacertus, the cells were rounded and disengaged from the glass; as a result, on the wall of the test tube there remained a dense network of anthrax chains, repeating the picture of a monolayer, and infrequent cells between them. Here we observed changes in the anthrax bacilli themselves; in the places of contact with the cells the bacilli swelled, became granular, and injury to the cell membrane was observed in a large part of them.

From what has been stated the conclusion can be made, that the anthrax bacilli exert a cytopathic effect on tissue cultures of human amnion and HEP2. In order to resolve the problem of the possibility of using these cultures for the identification of anthrax bacilli based on virulence it is necessary that there be further study of a large number of strains.